

cDNA SYNTHESIS PROTOCOL: INVITROGEN®

This protocol is adapted from an Invitrogen protocol by the Gene Expression Lab.

This protocol is for use with Invitrogen's Superscript™ III First Strand Synthesis Systems for RT-PCR Systems. For additional technical inquiries, contact Technical Service at 800-955-6288 or www.invitrogen.com

BEFORE STARTING THE EXPERIMENT cDNA SYNTHESIS PROTOCOL

Step A. Denature

Step B. Anneal

BEFORE STARTING THE EXPERIMENT:

Use PCR Hood

For ~30 minutes prior to starting procedure, use the UV light to decontaminate the hood.

Superscript III Reverse Transcriptase

Superscript III Reverse Transcriptase has been engineered to reduce Rnase activity and provide increased thermal stability. Other reverse transcriptases may be used; please contact Tech support at Invitrogen.

cDNA SYNTHESIS PROTOCOL

Step A: Denature

1. Remove RNA from -70°C , centrifuge for 20' at 10,000rpm at 4°C
2. Remove supernatant. Resuspend in 0.5ml of 75% ETOH
3. Spin sample for 20 minutes at 10,000rpm (4°C).
4. Remove supernatant and air dry the pellet
5. Resuspend RNA pellet in 50ul of DEPC water
6. Heat at 65°C for 3 minutes to denature RNA, briefly centrifuge and mix
7. Store on ice
8. Mix and briefly centrifuge each component before use.

9. In a 0.5ml tube combine (combine 1pg to 500ng of poly(a) RNA or 1pg to 5ug of total RNA into first-strand cDNA)

****For up to 5ug of total RNA use 1ul Primer****

<u>Component</u>	<u>1rxn</u>
50µm Oligo DT	1ul
RNA	nul
10mM dNTP Mix	1ul
DEPC-treated water	<u>nul</u>
FINAL VOLUME	10ul

3. Heat at **65°C for 5 min.** to denature RNA and primer. Place on ice for at least 1min.

Step B: cDNA Synthesis:

4. Prepare cDNA Synthesis Mix.
- Just before using, vortex the 10x RT Buffer for 5 seconds.
5. Prepare master mix on ice, adding each component in the indicated order.

<u>cDNA Synthesis Mix</u>	
10x RT Buffer	2
25mM MgCl ₂	4
0.1 M DTT	2
RNaseOUT	1
SuperScript III RT	<u>1</u>
	10ul

6. Add appropriate volume of master mix to each RNA/Primer Mixture. Mix gently, and collect by brief centrifugation. **Incubate at 50°C for 50 min.**
7. Terminate the reaction at 85°C **for 5 min.** Chill on ice.
8. cDNA reactions can be stored at -20°C or used for PCR immediately.